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File: USPT

Aug 28, 2001

US-PAT-NO: 6280994

DOCUMENT-IDENTIFIER: US 6280994 B1

TITLE: Zace 1: a human metalloenzyme

DATE-ISSUED: August 28, 2001

INVENTOR INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sheppard; Paul O.	Granite Falls	WA		

US-CL-CURRENT: 435/226; 435/252.3, 435/252.33, 435/320.1,
435/69.1, 435/69.7, 536/23.2, 536/23.4

Full	Title	Citation	Front	Review	Classification	Date	Reference
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DEPR:

A pair of nucleic acid molecules, such as DNA-DNA, RNA-RNA and DNA-RNA, can hybridize if the nucleotide sequences have some degree of complementarity. Hybrids can tolerate mismatched base pairs in the double helix, but the stability of the hybrid is influenced by the degree of mismatch. The $T_{sub.m}$ of the mismatched hybrid decreases by 1.degree. C. for every 1-1.5% base pair mismatch. Varying the stringency of the hybridization conditions allows control over the degree of mismatch that will be present in the hybrid. The degree of stringency increases as the hybridization temperature increases and the ionic strength of the hybridization buffer decreases. Stringent hybridization conditions encompass temperatures of about 5-25.degree. C. below the $T_{sub.m}$ of the hybrid and a hybridization buffer having up to 1 M Na^{sup.+}. Higher degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the $T_{sub.m}$ of the hybrid about 1.degree. C. for each 1% formamide in the buffer solution. Generally, such stringent conditions include temperatures of 20-70.degree. C. and a hybridization buffer containing up to 6.times.SSC and 0-50% formamide. A higher degree of stringency can be achieved at temperatures of from 40-70.degree. C. with a hybridization buffer having up to 4.times.SSC and from 0-50% formamide. Highly stringent conditions typically encompass temperatures of 42-70.degree. C. with a hybridization buffer having up to 1.times.SSC and 0-50% formamide. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes.

DEPR:

Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the properties of the chosen support.

See, for example, Affinity Chromatography: Principles & Methods (Pharmacia LKB Biotechnology 1988), and Doonan, Protein Purification Protocols (The Humana Press 1996).

DEPR:

Well-established hybridization methods of RNA detection include northern analysis and dot/slot blot hybridization (see, for example, Ausubel (1995) at pages 4-1 to 4-27, and Wu et al. (eds.), "Analysis of Gene Expression at the RNA Level," in Methods in Gene Biotechnology, pages 225-239 (CRC Press, Inc. 1997)). Nucleic acid probes can be detectably labeled with radioisotopes such as ³²P or ³⁵S. Alternatively, Zace1 RNA can be detected with a nonradioactive hybridization method (see, for example, Isaac (ed.), Protocols for Nucleic Acid Analysis by Nonradioactive Probes (Humana Press, Inc. 1993)). Typically, nonradioactive detection is achieved by enzymatic conversion of chromogenic or chemiluminescent substrates. Illustrative nonradioactive moieties include biotin, fluorescein, and digoxigenin.

DEPR:

Anti-Zace1 immunoconjugates can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody is determined by exposing the immunoconjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

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File: USPT

Aug 28, 2001

US-PAT-NO: 6280994

DOCUMENT-IDENTIFIER: US 6280994 B1

TITLE: Zace 1: a human metalloenzyme

DATE-ISSUED: August 28, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sheppard; Paul O.	Granite Falls	WA		

US-CL-CURRENT: 435/226; 435/252.3, 435/252.33, 435/320.1,
435/69.1, 435/69.7, 536/23.2, 536/23.4

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 2. Document ID: US 6258559 B1

L8: Entry 2 of 2

File: USPT

Jul 10, 2001

US-PAT-NO: 6258559

DOCUMENT-IDENTIFIER: US 6258559 B1

TITLE: Method for producing proteins in transformed Pichia

DATE-ISSUED: July 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zamost; Bruce L.	Seattle	WA		

US-CL-CURRENT: 435/69.1

Full	Title	Citation	Front	Review	Classification	Date	Reference
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File: USPT

Jul 10, 2001

DOCUMENT-IDENTIFIER: US 6258559 B1

TITLE: Method for producing proteins in transformed Pichia

DEPR:

These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, Affinity Chromatography: Principles & Methods (Pharmacia LKB Biotechnology 1988), and Doonan, Protein Purification Protocols (The Humana Press 1996).

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